DRUG DISCOVERY

Edward A. Sausville, M.D., Ph.D.
Professor of Medicine
Associate Director for Clinical Research
Marlene & Stewart Greenebaum Cancer Center
University of Maryland at Baltimore
March 19, 2009

OUTLINE OF PRESENTATION

- ***General Introduction***
- Definition of Drug Targets
- Generating Diversity
- Definition of Lead Structures
- Qualifying Leads for Transition to Early Trials

DRUG DISCOVERY: WHERE HAS IT WORKED?

Majority of Drug Targets:	% Top Sales
- G-Protein Coupled Receptors	18
- Nuclear (Hormone) Receptors	10
- Ion Channels	16
- Enzymes	~50

Problem:

How to choose target likely to succeed especially if directed at new target (e.g. protein-protein interactions)?

Nature 384 suppl 11:5, 1996

DRUG DISCOVERY: A SUCCESSION OF STYLES

Antiquity to 1960s:
Mixtures of natural products vs. bioassays
(e.g., digitalis, rauwolfia, penicillins, anthracyclines, vinca, taxol, camptothecins)

1930s to present:
Pure compounds vs. bioassays
(e.g., sulfas, diuretics, hypoglycemics, antiHBP)

1960s to present:
Pure compounds vs. pure enzymes
(e.g., ACE inhibitors, cholesterol-lowering statins, RT and protease inhibitors)

1980s to present:
Combinatorial methods to bring mixtures of compounds vs. many targets

WHY COMPOUNDS FAIL AND SLOW DOWN IN DEVELOPMENT

Reasons for failure

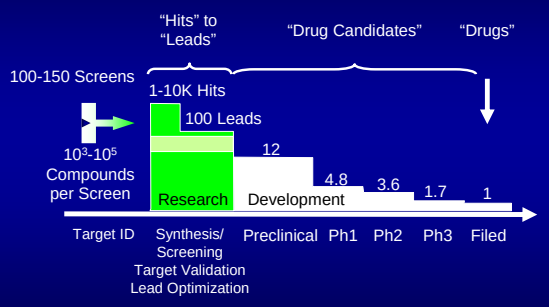
- Toxicity, 22%
- Lack of efficacy, 31%
- Market reasons, 6%
- Poor biopharmaceutical properties, 41%

Reasons for slowdown

- Synthetic complexity
- Low potency
- Ambiguous toxicity finding
- Inherently time-intensive target indication
- Poor biopharmaceutical properties

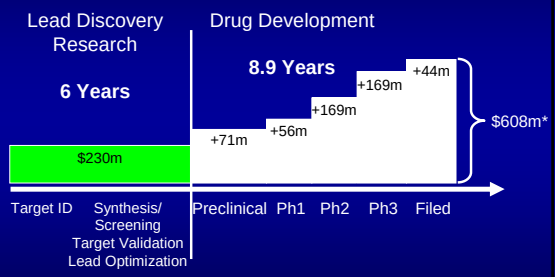
Modern Drug Discovery
January/February 1999
Modern Drug Discovery, **1999**, *2 (1)*, 55-60.

TRADITIONAL PHARMACEUTICAL R&D Suffers High Attrition*



** Tufts CSDD, H&Q 1998; The Pfizer Journal, 1/2000*

TRADITIONAL PHARMACEUTICAL R&D
Costly* and Time Consuming**



** Lehman Brothers, 1997; ** Tufts CSDD*

OUTLINE OF PRESENTATION

- General Introduction
- ***Definition of Drug Targets***
- Generating Diversity
- Definition of Lead Structures
- Qualifying Lead for Transition to Early Trials

TWO CONTRASTING DRUG-DISCOVERY "PHILOSOPHIES"

- "EMPIRICAL": Recognize initial drug lead by functionally useful effect
  -E.g. : penicillin (anti-bacterial effect)
  rauwolfia (anti-hypertensive)
  taxol (anti-tumor)
  digoxin (cardiotonic / antiarrythmic)
- "RATIONAL": Recognize drug by design or screen against biochemical target's function
  -E.g.: HIV-protease inhibitor (anti-infection)
  metoprolol (anti-hypertensive)
  methotrexate (anti-tumor)

"EMPIRICAL" DRUG DISCOVERY

SCREEN → BIOLOGICAL ACTIVITY (*in vitro/in vivo*) ↔ CHEMISTRY ↔ PHARMACOLOGY

↓

OPTIMIZED SCHEDULE (*in vivo*)

↓

IND-DIRECTED TOX/FORMULATION

↓

PHASE I: DOSE/SCHEDULE HUMAN PHARM/TOX

↓

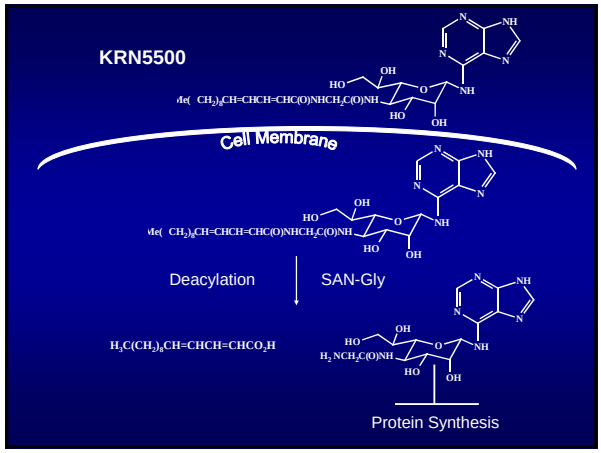
PHASE II: ACTIVITY

↓

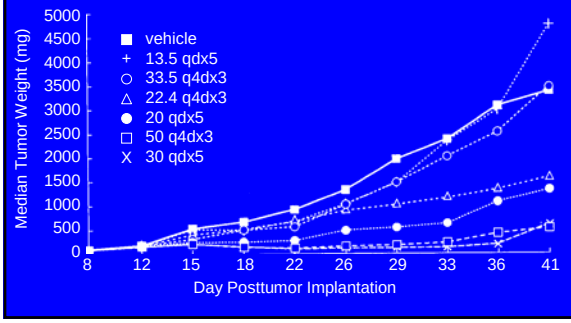
PHASE III: COMPARE WITH STANDARD

PROBLEMS WITH EMPIRICAL MODELS

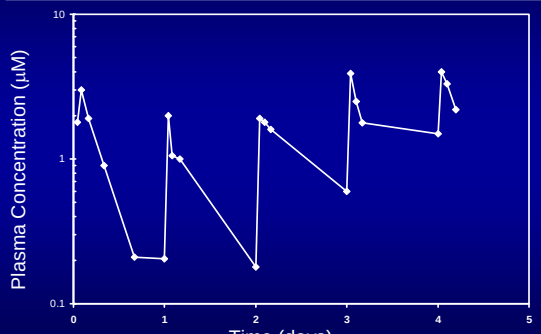
- Lead optimization difficult without known biochemical target--How to optimize?
- Value of screen depend on predictive value of screening model with biology of disease
  - -E.g.: acid hypo-secretion or H2 receptor binding assay HIGHLY correlate with useful anti-ulcer Rx
  - -Counter E.g.: anitumor activity in > 33% mouse models of cancer have at best 50% chance of >1 P2 trial for non=targeted cancer Rx's
- Divorced from mechanism: an intriguing lead must be "deconvolutedh



EFFECT OF KRN5500 ON COLO-205 ATHYMIC MOUSE XENOGRAFTS



KRN5500 PLASMA CONCENTRATIONS ON EFFECTIVE SCHEDULE(20 MG/KG/D) IN MICE



SUMMARY OF KRN-5500 PHASE I

- 26 patients as IV once per day over 5 days
- Dose limiting toxicity = interstitial pneumonitis
- MTD = 2.9 mg/M²/d x 5
- Achieve only 0.75 - 1 μM at 3.7 mg/M²/d x 5
- 4/6 patients with >25% incr C_{max} have grade 4 toxicity

Data of J. P. Eder, DFCI

"RATIONAL" DRUG DISCOVERY

MOLECULAR TARGET SCREEN ⟷ PHARMACOLOGY (to affect target)

Biochemical
Engineered cell
Animal (yeast/worm/fish)

CHEMISTRY

TARGET-DEPENDENT *IN VIVO* MODEL

IND DIRECTED TOX/FORM

PHASE I: DOSE/SCHEDULE: HUMAN PHARM/TOX;
? AFFECT TARGET

PHASE II: ACTIVITY = ? AFFECT TARGET

PHASE III: COMPARE WITH STANDARD; STRATIFY BY TARGET?

bcr-abl AS TARGET: RATIONALE

- Apparently pathogenetic in t9:Q22 (Ph+) CML/ALL
- Absence in normal tissues
- Modulate signal transduction events downstream
  - Maintenance of chronic phase
  - Adjunct to bone marrow transplantation

bcr-abl FUSION PROTEIN

bcr

autophosphorylation

Phosphorylation of other substances

McWhirter JR, EMBO *12:1533, 1993*

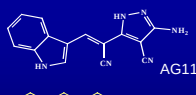
EXAMPLE OF "RATIONAL" APPROACH: bcr-abl directed agents

Natural product empiric lead

erbstatin lavendustin piceatannol

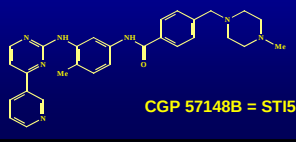
1st generation synthetic

AG957



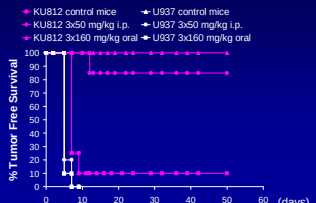
AG1112

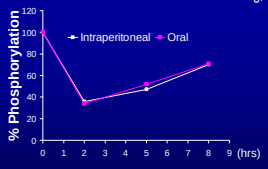
2nd generation synthetic; in clinic

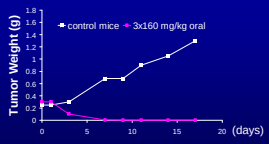


CGP 57148B = STI571

STI571: An oral in vivo bcr-abl kinase inhibitor







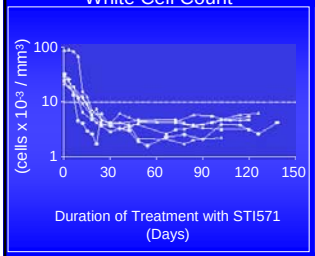
Tyr phosphorylation *in vivo* **Antitumor activity *in vivo***

le Coutre et al, JNCI 91:163, 1999

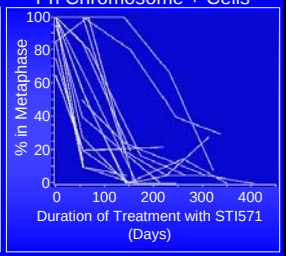
EFFICACY AND SAFETY OF A SPECIFIC INHIBITOR OF THE BCR-ABL TYROSINE KINASE IN CHRONIC MYELOID LEUKEMIA

BRIAN J.DRUKER,M.D.,MOSHE TALPAZ,M.D.,DEBRA J.RESTA,R.N.,BIN PENG,PH.D.,
ELISABETH BUCHDUNGER,PH.D.,JOHN M.FORD,M.D.,NICHOLAS B.LYDON,PH.D.,HAGOP KANTARJIAN,M.D.
RENAUD CAPDEVILLE,M.D.,SAYURI OHNO-JONES,B.S.,AND CHARLES L.SAWYERS,M.D.

White Cell Count



Ph Chromosome + Cells

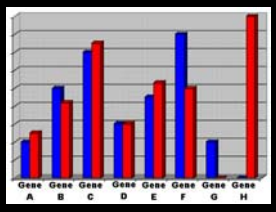


NEJM 344: 1031, 2001

MOLECULAR TARGET DEFINITION - HOW TO?

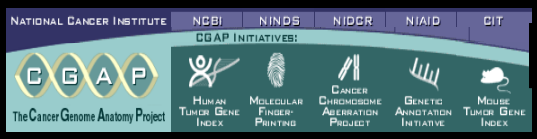
- **BIOLOGY:**
  - * Cytogenetics ⟶ Breakpoints ⟶ Molecules (bcr-abl)
  - * "Positive" selection from tumor DNA ⟶ Active oncogenes (signal transduction)
  - * Tumor gene expression profiling (CGAP)
- **" RETROFIT" ACTIVE MOLECULES:**
  - * Binding partners (geldanamycin, rapamycin, fumagillin)
  - * Computational algorithm (molecule ⟷ target)
    - COMPARE
    - Cluster analysis
- **"CLASSICAL:"**
  - * Cell metabolism / Biochemistry
  - * Suggest single targets ⟶ Inefficient; Medicinal Chemistry possible
- **CHEMICAL GENETICS:**
  - * Libraries of molecules and precisely defined organisms

Gene Expression: The Cell's Fingerprint



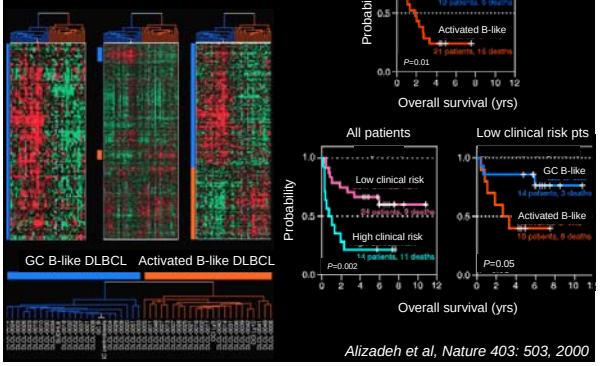
Establishing for a cell the repertoire of genes expressed, together with the amount of gene products produced for each, yields a powerful "fingerprint". Comparing the fingerprints of a normal versus a cancer cell will highlight genes that by their suspicious absence or presence (such as Gene H) deserve further scientific scrutiny to determine whether such suspects play a role in cancer, or can be exploited in a test for early detection.

http://cgap.nci.nih.gov



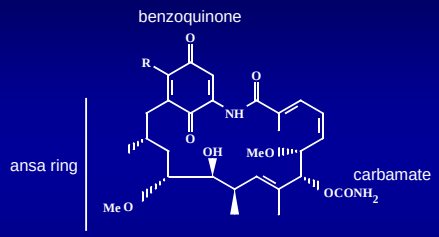
http://cgap.nci.nih.gov

Distinct types of diffuse large B-cell lymphoma identified by gene expression profiling



Alizadeh et al, Nature 403: 503, 2000

GELDANAMYCIN: EXAMPLE OF BINDING PARTNER DEFINING TARGET



	NSC	R
Geldanamycin	122750	OMe
17-AAG	330507	$NHCH_2CH=CH_2$

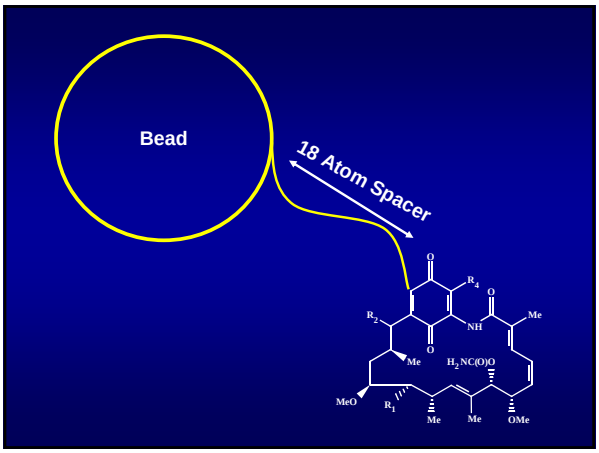
BENZOQUINOID ANSAMYCINS INITIAL CELL PHARMACOLOGY - I

- "Reverse" transformed phenotype of src-transformed rat kidney cell line
  - decrease tyrosine phosphorylation of pp60src
  - not inhibit pp60 immune complex kinase directly but these were inhibited from drug-treated cells
  - thus alter "intracellular environment" of src

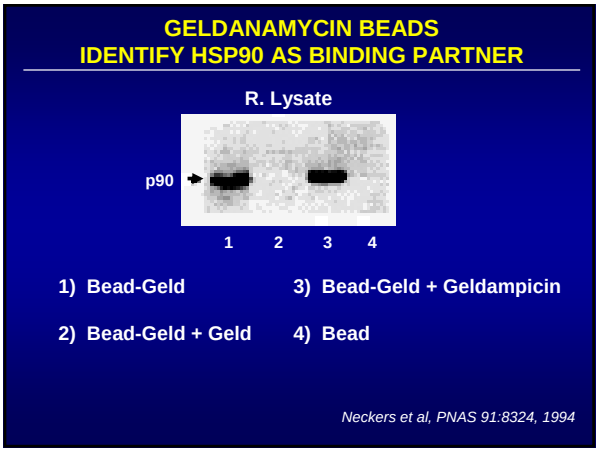
  (Uehara et al, MCB 6: 2198, 1986)

- Decrease steady state phosphorylation levels to 10% of control
  - decrease steady state level of pp60src by 30%
  - accelerate turnover of pp60src

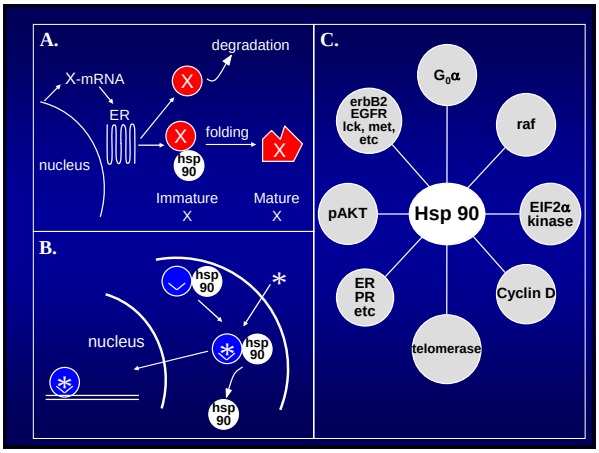
  (Uehara et al, Cancer Res 49: 780, 1989)



GELDANAMYCIN BEADS IDENTIFY HSP90 AS BINDING PARTNER



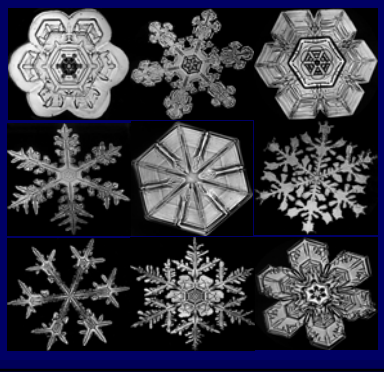
Neckers et al, PNAS 91:8324, 1994



OUTLINE OF PRESENTATION

- General Introduction
- Definition of Drug Targets
- ***Generating Diversity***
- Definition of Lead Structures
- Qualifying Lead for Transition to Early Trials

Diversity



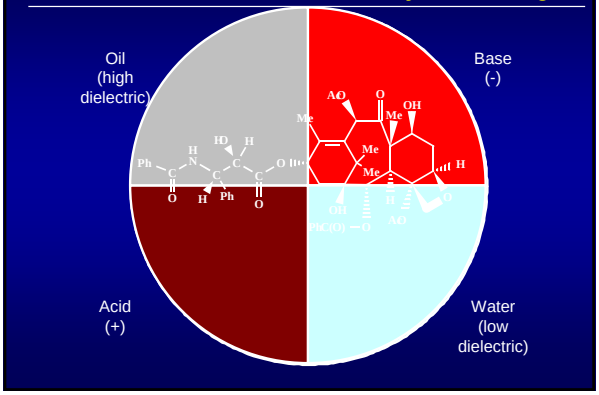
It is estimated that there are 10^{40} compounds in all of "chemical space". Since the Big Bang, there have only been 10^{17} seconds.

- Peter Wipf

SOURCES OF DIVERSITY

- "Natural Products" = entities derived from plants, animals, bacteria, etc. May have "ethnopharmacognosy" to suggest use
  - "pure compound" collections
  - extracts: aqueous/organic
  - genetically altered producer organisms
- Target non-selected chemical compound libraries
  - peptide / protein
  - non-peptide
- Target-directed chemical compound libraries
  - "classical" medicinal chemistry / bona fide crystal structure - derived
  - "docked" lead structures into model

Natural Products: Unique arrays of the four "elements" which make a really useful drug



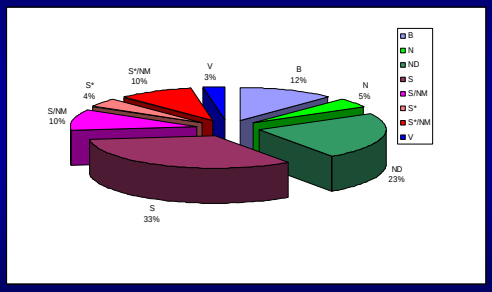
Sources of "Modern Drugs"

If one looks at the current drug scene from a chemical perspective (data from 1981 – 2002) then the following slides show reasonable approximations of the sources of drugs currently approved, World-wide, by the FDA or equivalent body.

Codes are:

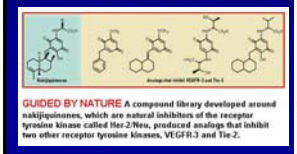
N	Natural Product
ND	Natural Product Derivative
S*	Natural Product Pharmacophore
S	Synthetic Compound
B/V	Biological / Vaccine
(NM)	Natural Product Mimic as a subdivision

Sources of Drugs (1981-2002); Extended Subdivisions n = 1031

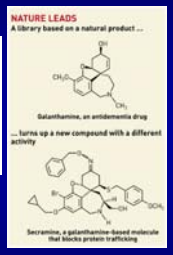


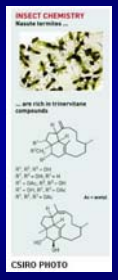
Newman et al, J. Nat. Prod., 2003, 66, 1027-1037

EXAMPLES OF NP LEAD GENERATION OF NOVEL SCAFFOLDS



GUIDED BY NATURE A compound library developed around nakijiquinones, which are natural inhibitors of the receptor tyrosine kinase called Her-2/Neu, produced analogs that inhibit two other receptor tyrosine kinases, VEGFR-3 and Tie-2.



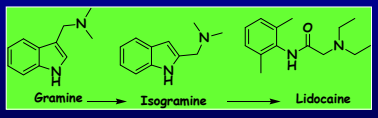


Discovery of Lidocaine

*Central Asian camels refused to eat a certain type of reed

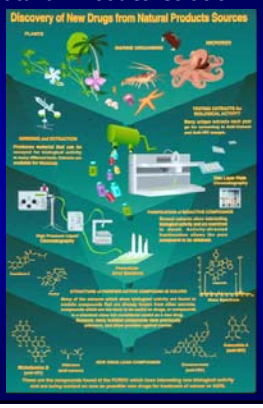
*Characterization of gramine as the antifeedant principle led to the synthesis of isogramine

*Taste-test: numbness; therefore, lead for anesthetic agent development

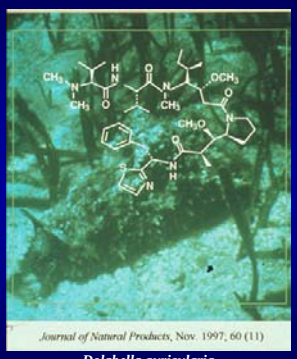


Courtesy of N. R. Farnsworth

Natural Product Isolation Tree



"You are what you eat"



Dolabella auricularia

Dolastatins come from a *Symploca* species that they graze on

"Non-culturable" versus "Cultured" microbes

- **The microbial World has only just been scratched.**
  - **Much less than 1% of the available organisms have even been seen, let alone identified.**
- **In soil, there are estimates of > 1000 species per gram**
  - **very few can be cultured**
  - **these may not be representative of the "Soil meta-Genome"**
- **Over 1000 microbes per mL of seawater can be seen and only ~ 1% can be cultured using current methods.**

SOURCES OF DIVERSITY

- "Natural Products" = entities derived from plants, animals, bacteria, etc. May have "ethnopharmacognosy" to suggest use
  - "pure compound" collections
  - extracts: aqueous/organic
  - genetically altered producer organisms
- Target non-selected chemical compound libraries
  - peptide / protein
  - non-peptide
- Target-directed chemical compound libraries
  - "classical" medicinal chemistry / bona fide crystal structure - derived
  - "docked" lead structures into model

TRIPEPTIDE COMBINATORIAL LIBRARY

X X X

Four amino acids in each position
$4^3 = 64$

A = Alanine
R = Arginine
T = Threonine
W = Tryptophan

after R. Houghten, 1999

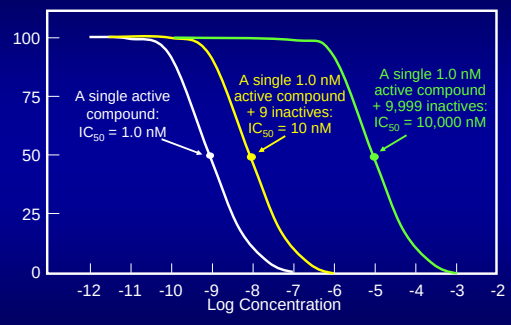
NUMBER OF PEPTIDES POSSIBLE WITH INCREASING LENGTH

Length	Peptide	Number
2	Ac – OO – NH_2	400
3	Ac – OOO – NH_2	8,000
4	Ac – OOOO – NH_2	160,000
5	Ac – OOOOO – NH_2	3,200,000
6	Ac – OOOOOO – NH_2	64,000,000
7	Ac – OOOOOOO – NH_2	1,280,000,000
8	Ac – OOOOOOOO – NH_2	25,600,000,000

O = Individual Defined Amino Acid

after R. Houghten, 1999

IC_{50} OF MIXTURES

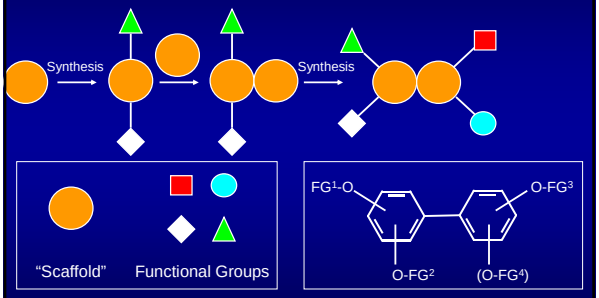


COMBINATORIAL LIBRARIES: THE MIXTURE QUESTION

	Natural Product Extracts	Synthetic Combinatorial Mixtures
Direct screening of compound mixtures	Yes	Yes
Discovery of highly active compounds	Yes	Yes
Equal concentrations of compounds	No	Yes
Chemical structures known	No	Yes
Synthetic pathway known	No	Yes
Structure – activity relationship known	No	Yes

after R. Houghten, 1999

NON-PEPTIDE "COMBINATORIAL" STRATEGIES COMBINE "SCAFFOLDS" (OR "BACKBONES") WITH "FUNCTIONAL GROUPS"



The Chemical Generation of Molecular Diversity from
http://www.netsci.org/Science/Combichem/feature01.html

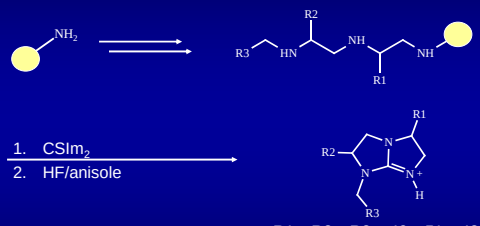
THE RULE OF FIVE

An awareness tool for discovery chemists:
Compounds with two or more of the following characteristics are flagged as likely to have poor oral absorption

- More than 5 H-bond donors
- Molecular weight >500
- c log P > 5
- Sum of N's and O's (a rough measure of H-bond acceptors) > 10

Modern Drug Discovery
January/February 1999
Modern Drug Discovery, **1999**, 2 *(1)*, 55-60.

COMBINATORIAL LIBRARIES OF BICYCLIC GUANIDINES FROM REDUCED ACYLATED DIPEPTIDES



1. $CSIm_2$
2. HF/anisole

R1 x R2 x R3 = 49 x 51 x 42 = 104,958 compounds

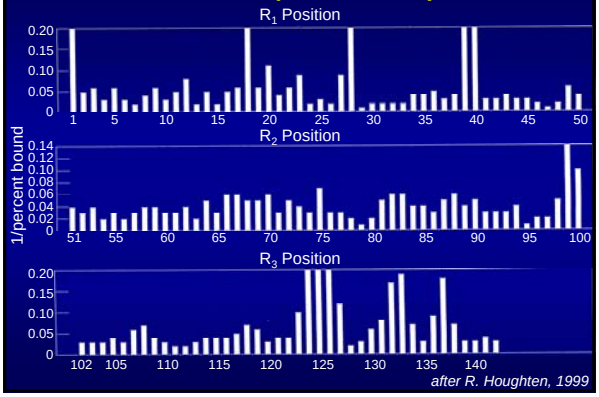
after R. Houghten, 1999

BIOASSAYS (READY APPLICATION OF SOLUBLE LIBRARIES)

- Soluble Acceptors
  - antibodies
  - enzymes
- Membrane-bound Receptors
  - tissue homogenate
  - functional cell based
- Microorganisms: Disruption of Function
  - bacteria
  - fungi
  - virus
- Differentiation
  - stem cells
- *In Vivo*

after R. Houghten, 1999

POSITIONAL SCANNING BICYCLIC GUANIDINE LIBRARY (κ RECEPTOR)



after R. Houghten, 1999

OUTLINE OF PRESENTATION

- General Introduction
- Definition of Drug Targets
- Generating Diversity
- ***Definition of Lead Structures***
- Qualifying Lead for
- Transition to Early Trials

" RATIONAL":
-***Structure based design***
-Biochemical Screen
-Target-driven
Cell-based Screen
"EMPIRICAL"
-Bioassay of effect

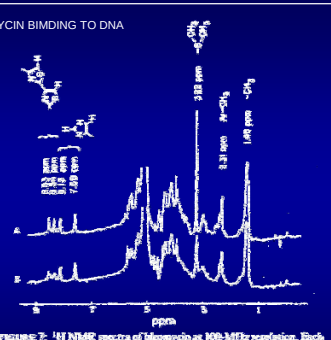
NMR-BASED SCREENING

1. Screen “fragment” like molecules with “leadlike” properties (MW <300; ClogP ~1.5)
2. Characterize ***binding*** and portion of molecule to which they bind
3. Ligands with weak affinities can be defined (~K_D = 5mM)
4. Lead to high affinity binders through iterative screening
5. Can label protein of interest with isotopes “sensitive” to ligand effects (e.g. N15) and utilize proton resonances of drug to simultaneously allow definition of ligand and receptor binding sites

Hajduk et al. J Med Chem 48: 2518, 2005

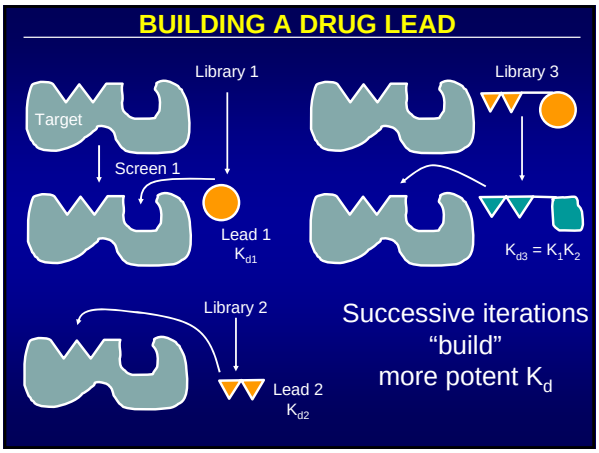
NMR AS MEANS OF DEFINING BINDING SITES

E.G., BLEOMYCIN BIMDING TO DNA



Horwitz et al. Biochemistry 16: 3641, 1977

BUILDING A DRUG LEAD

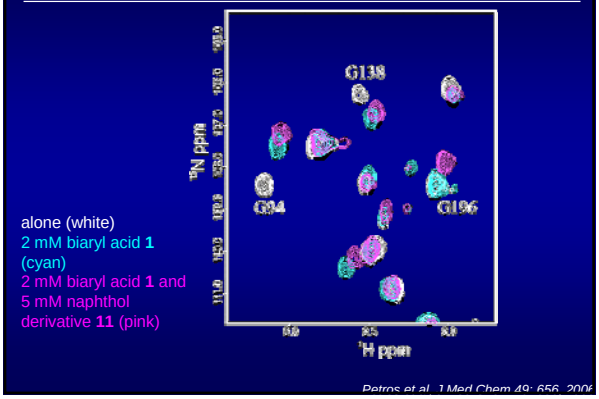


AFFINITIES OF SELECTED BIARYL COMPOUNDS FOR BCL-XL

No.	Structure	NMR K_d (μM)	No.	Structure	NMR K_d (μM)
1		390 ± 30	11		4300 ± 1600
2		1300 ± 530	12		12000 ± 7000
3		> 5000	13		5800 ± 2800
4		> 5000	14		2900 ± 440
5		> 5000	15		11000 ± 4000
6		2000 ± 1000	16		12000 ± 4500
7		1000 ± 300	17		5000 ± 2000
8		300 ± 117	18		4000 ± 2000
9		250 ± 100	19		6000 ± 2000
10		250 ± 100	20		6000 ± 2000

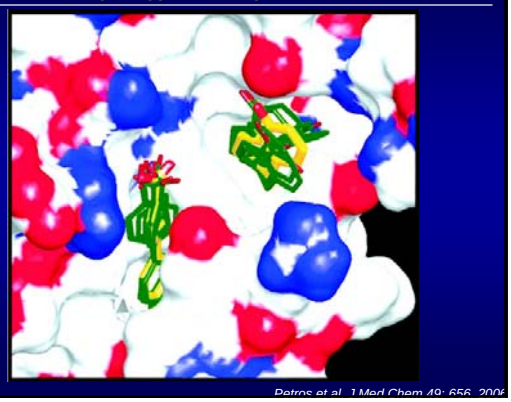
Petros et al. J Med Chem 49: 656, 2006

SECTION FROM A ^{15}N HSQC SPECTRUM OF BCL-XL IN THE PRESENCE AND ABSENCE OF COMPOUND



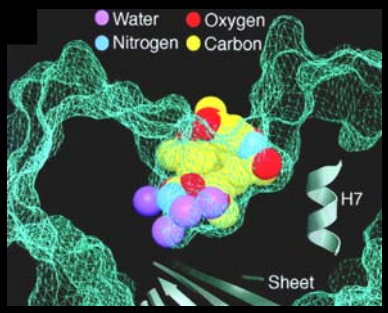
Petros et al. J Med Chem 49: 656, 2006

SUPERPOSITION OF SEVEN LOW-ENERGY STRUCTURES CALCULATED FOR BCL-XL COMPLEXED TO 1 AND 11



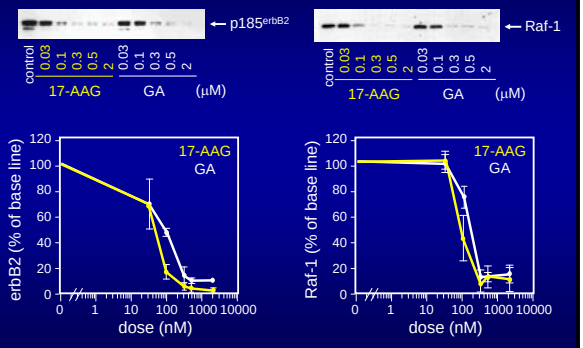
Petros et al, J Med Chem 49: 656, 2006

THREE DIMENSIONAL VIEW OF GELDANAMYCIN BINDING POCKET IN AMINO TERMINUS OF HSP90



Stebbins et al, Cell 89:239, 1997

17-AAG BINDS TO HSP90 & SHARES IMPORTANT BIOLOGIC ACTIVITIES WITH GELDANAMYCIN



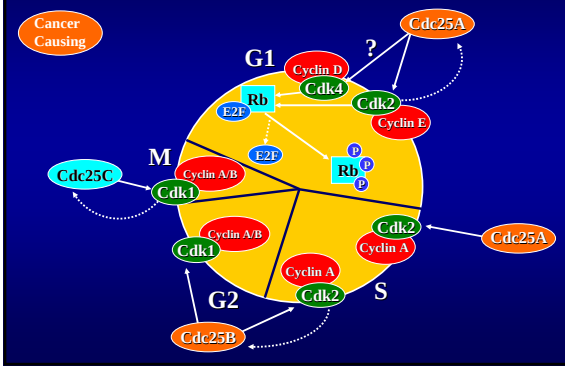
Schulte & Neckers, Cancer Chemother Pharmacol 42: 273, 1998

OUTLINE OF PRESENTATION

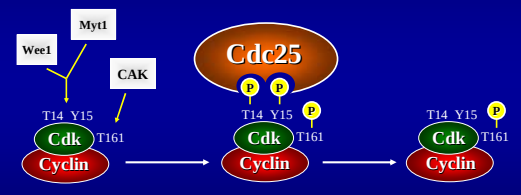
- General Introduction
- Definition of Drug Targets
- Generating Diversity
- ***Definition of Lead Structures***
- Qualifying Lead for
- Transition to Early Trials

" RATIONAL":
-Structure based design
-*Biochemical Screen*
-Target-driven
Cell-based Screen
"EMPIRICAL"
-Bioassay of effect

Cell cycle regulation by Cdc25 phosphatases



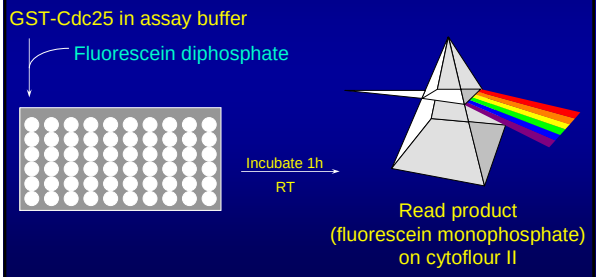
Regulation of Cell Cycle Progression by Cdc25: Cdk Activation



CDC25 Phosphatases and Cancer

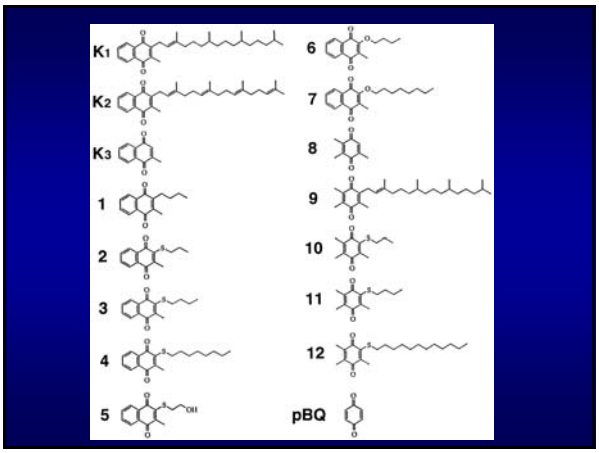
- CDC25A and B overexpressed in many cultured cancer cell lines.
- Cdc25A suppresses apoptosis.
- Overexpression of CDC25A or B has been detected in human breast, head and neck, cervical, skin, lymph, lung and gastric cancers.
- Human CDC25A & B cooperated with Ha-Ras^{G12V} and CDC25A cooperated with Rb $^{-/-}$ in the oncogenic focus transformation of mouse embryonic fibroblasts and tumor formation in nude mice. Thus, Cdc25A & B may be human oncogenes.

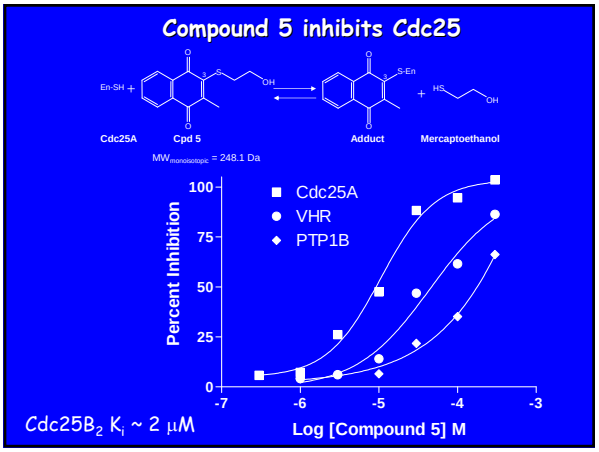
Method for identifying Cdc25 phosphatase inhibitors

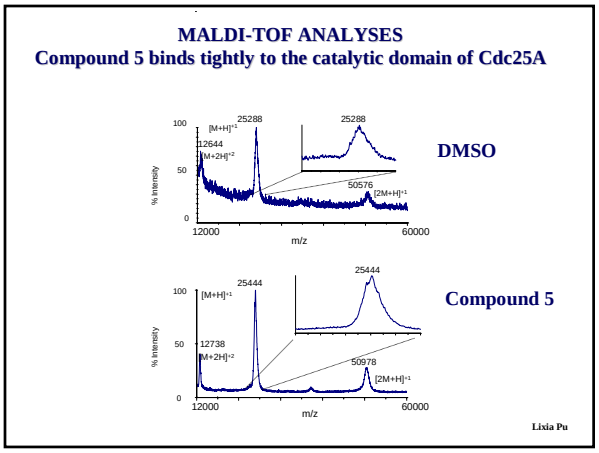


Chemical Screening Approach

- Targeted Array Libraries
- Diverse Chemical Libraries


K1
K2
K3
1
2
3
4
5
6
7
8
9
10
11
12
pBQ

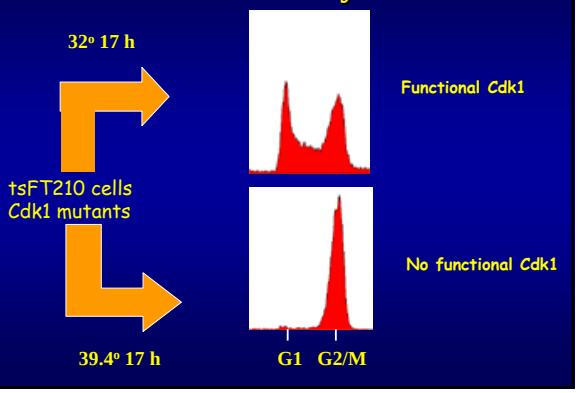

Compound 5 inhibits Cdc25
En-SH +
OH
S-En
HS
OH
Cdc25A
Cpd 5
Adduct
Mercaptoethanol
MW monoisotopic = 248.1 Da
Percent Inhibition
100
75
50
25
0
-7
-6
-5
-4
-3
Cdc25A
VHR
PTP1B
Log [Compound 5] M
Cdc25B2 Ki ~ 2 μM


MALDI-TOF ANALYSES
Compound 5 binds tightly to the catalytic domain of Cdc25A
25288
[M+H]+1
12644
[M+2H]+2
50576
[2M+H]+1
% Intensity
m/z
12000
60000
DMSO
25444
[M+H]+1
12738
[M+2H]+2
50978
[2M+H]+1
Compound 5
Lixia Pu

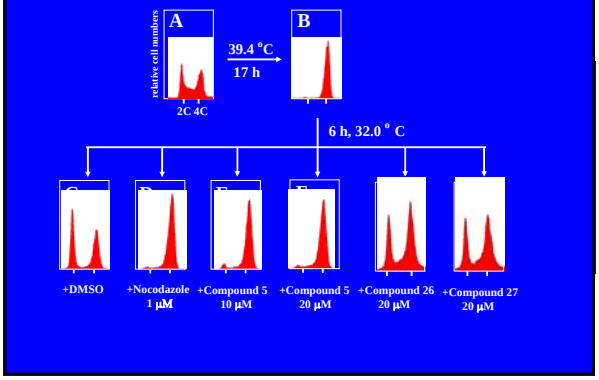
Compound Validation

- Cellular: Cell Cycle
- Biochemical: Substrate phosphorylation
- Genetic: Chemical complementation

tsFT210 Cell System



Compound 5 causes G2/M arrest



OUTLINE OF PRESENTATION

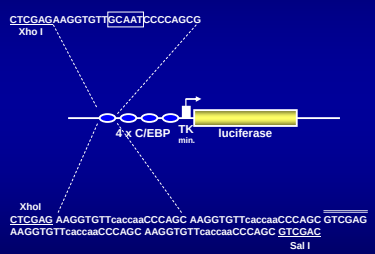
- General Introduction
- Definition of Drug Targets
- Generating Diversity
- ***Definition of Lead Structures***
- Qualifying Lead for
- Transition to Early Trials

" RATIONAL":
-Structure based design
-Biochemical Screen
-Target-driven
Cell-based Screen
"EMPIRICAL"
-Bioassay of effect

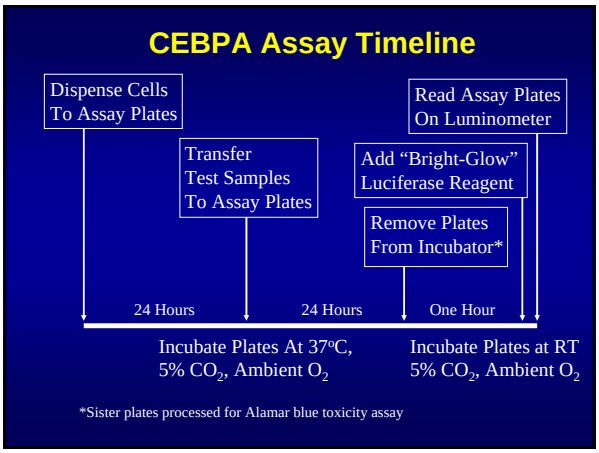
C/EBPα AS A TARGET FOR DEVELOPMENT OF NOVEL CANCER THERAPEUTICS

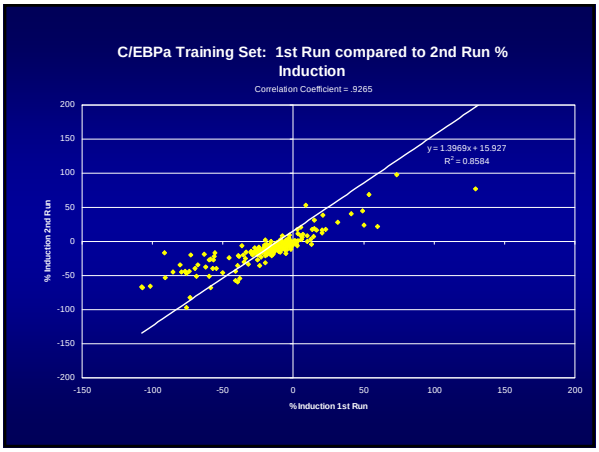
- **The transcription factor C/EBPα plays key roles in regulation of differentation of various cell lineages (adipocytes, keratinocytes, etc.)**
- **Mutations in CEBPA (the gene coding for C/EBPα) are associated with development of AML [t(8;21) - subtypes M1 and M2]**
- **CEBPA knock-out mice show no mature neutrophils**
- **Conditional expression of CEBPA is sufficient to trigger neutrophilic differentiation**
- **Pharmacologic modulators of CEBPA could act as differentiation inducers and thus limit proliferation of AML cells**

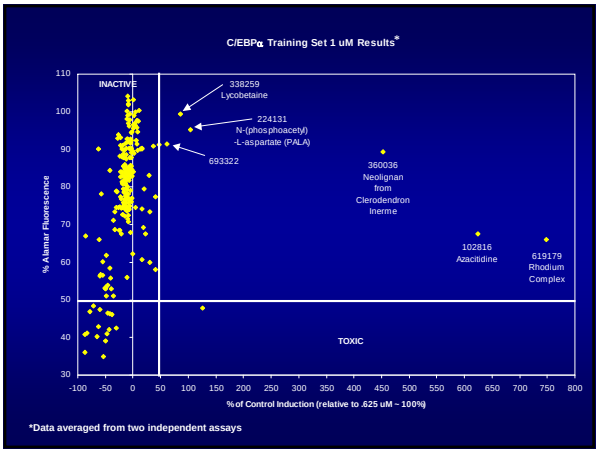
CEBP Reporter Construct*

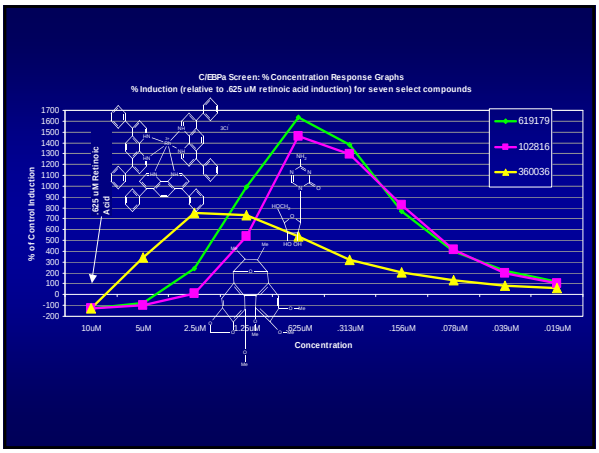


*Host cell for this construct is U-937


CEBPA Assay Timeline
Dispense Cells To Assay Plates
Transfer Test Samples To Assay Plates
Remove Plates From Incubator*
Add "Bright-Glow" Luciferase Reagent
Read Assay Plates On Luminometer
24 Hours
24 Hours
One Hour
Incubate Plates At 37°C, 5% CO2, Ambient O2
Incubate Plates at RT 5% CO2, Ambient O2
*Sister plates processed for Alamar blue toxicity assay

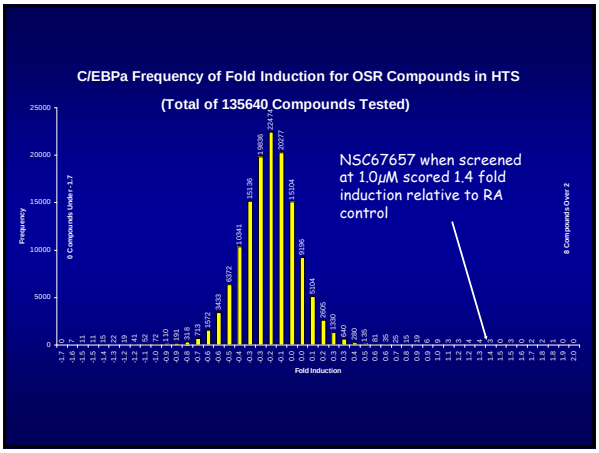

C/EBPa Training Set: 1st Run compared to 2nd Run % Induction
Correlation Coefficient = .9265
y = 1.3969x + 15.827
R2 = 0.8584
% Induction 2nd Run
% Induction 1st Run

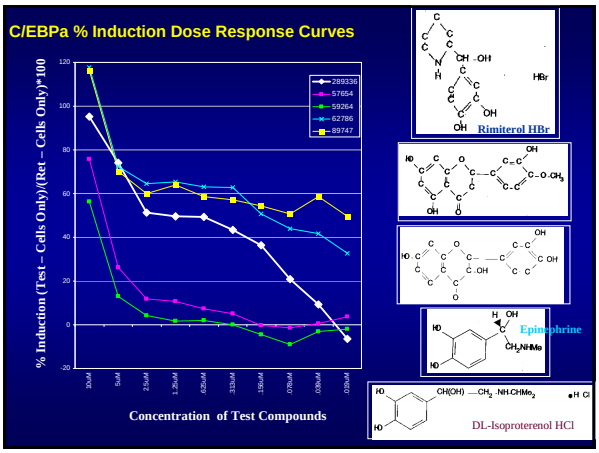

C/EBPα Training Set 1 uM Results*
INACTIVE
338259 Lycobetaine
224131 N-(phosphoacetyl) -L-aspartate (PALA)
693322
360036 Neolignan from Clerodendron Inerme
102816 Azacitidine
619179 Rhodium Complex
TOXIC
% Alamar Fluorescence
% of Control Induction (relative to .625 uM ~ 100%)
*Data averaged from two independent assays



Categories of Confirmed Actives in CEPBα HTS

- β-adrenergic agonists
- Toxic compounds (stress signaling)
- Retinoids
- HDAC Inhibitors
- Novel Drug Lead - Sterol mesylate

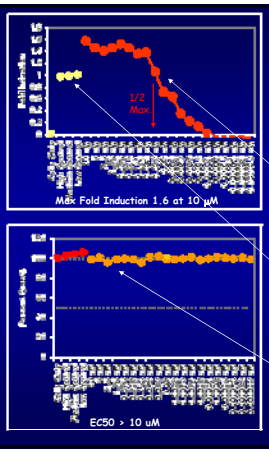




NSC 67657, a novel sterol mesylate inducer of CEBPα with potential anti-leukemic activity

Basis for Interest

- Identified in a DTP high-throughput screen of > 140,000 compounds
- Induced CEBP-luciferase activity at low concentrations: 50% activation at 40 nM
- Induced differentiation in U937cells as measured by CD11b or CD11c antigens or NBT staining
- Induced morphologic differentiation in HL60 cells
- Induced cell surface markers of monocytic differentiation in AML patient blasts ex vivo



Secondary testing of NSC67657 in C/EBPa (U937) cells

Dose-dependent increase of luciferase reporter activity (max. 1.6 fold)

Based on control induction of retinoic acid (1µM)

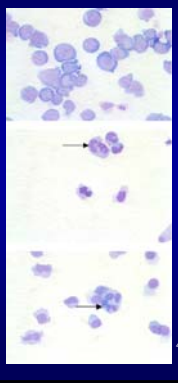
Activity occurs at non-toxic concentrations

Evidence for Morphologic Differentiation in HL60 Cells

Untreated control - largely myeloblasts

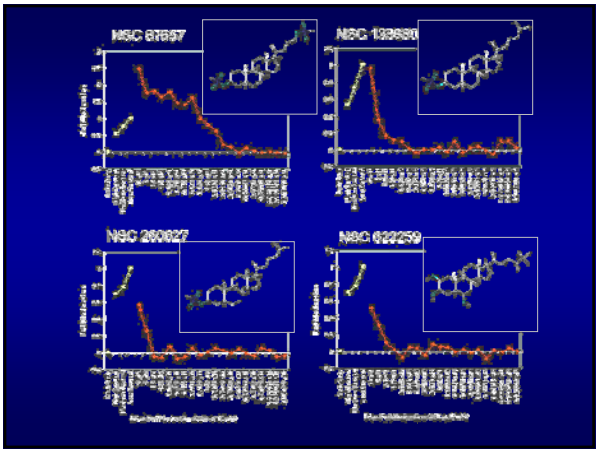
1 mM ATRA – Reduced cell numbers, segmented and cells resembling neutrophils (arrow)

20 mM NSC 67657 – Reduced cell numbers, segmented and cells resembling neutrophils (arrow)

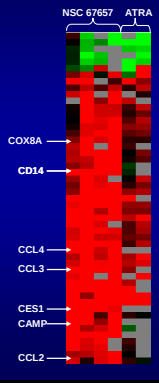


GENERATION OF SAR AROUND STEROID MESYLATE LEAD

- Related compounds available from the DTP Repository were tested in concentration-response format
- No compounds with comparable activity were found (most were completely inactive)
- Three compounds which showed some activity provided an initial SAR model



Hierarchical cluster of 51 genes dysregulated >3 fold over control by NSC 67657 in HL60 cells.



120h incubation

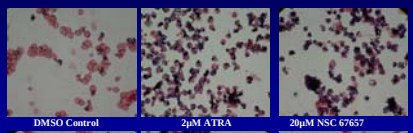
Key:
- up regulated gene
- down regulated gene
- no change
- unexpressed gene or missing data point

COX8A: cytochrome c oxidase subunit 8A
CD14 : surface protein preferentially expressed on monocytes/macrophages.
CCL4: chemokine (C-C motif) ligand 4;macrophage inflammatory protein
CCL3: chemokine (C-C motif) ligand 3; macrophage inflammatory protein.
CES1: carboxylesterase 1 (monocyte/macrophage serine esterase 1)
CAMP: cathelicidin antimicrobial peptide
CCL2: chemokine (C-C motif) ligand 4;macrophage inflammatory protein

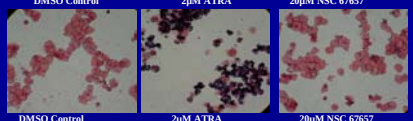
When compared to ATRA treated cells, several genes of the monocyte/ macrophage lineage were uniquely up regulated by NSC 67657.

NSC 67657 induces differentiation in different cell lines compared to ATRA

HL60 cells:
Can differentiate to either granulocytes or monocyte/macrophages



NB4 cells:
Can only differentiate into granulocytes

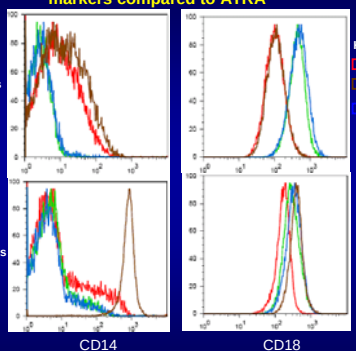


ATRA induces differentiation (measured by NBT reduction after 7 days) in both HL60 and NB4 cell lines, while NSC 67657 induced differentiation only in HL60 cells. This supports the monocyte/ macrophage lineage specific differentiation proposed from the gene expression studies

NSC 67657 induces a different pattern of cell surface markers compared to ATRA

NB4 cells

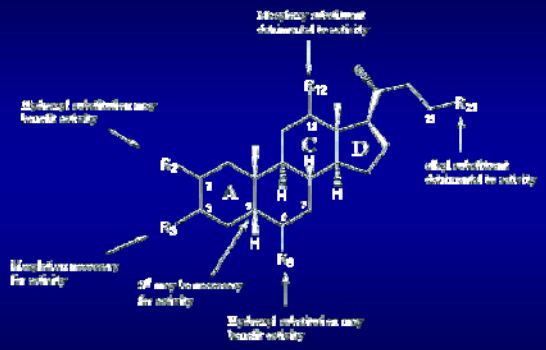
HL60 cells



Key:
- Control (DMSO)
- NSC 67657 (20μM)
- & ATRA (2 &10μM)

NSC 67657 induced CD14 expression only in HL60, not NB4 cells.
ATRA does not induce CD14 expression in either cell line (5 day incubation).

INITIAL STRUCTURE-ACTIVITY MODEL



OUTLINE OF PRESENTATION

- General Introduction
- Definition of Drug Targets
- Generating Diversity
- ***Definition of Lead Structures***
- Qualifying Lead for
- Transition to Early Trials

" RATIONAL":
-Structure based design
-Biochemical Screen
-Target-driven
Cell-based Screen
"EMPIRICAL"
-Bioassay of effect

NCI IN VITRO DRUG SCREEN

1985 Hypothesis:

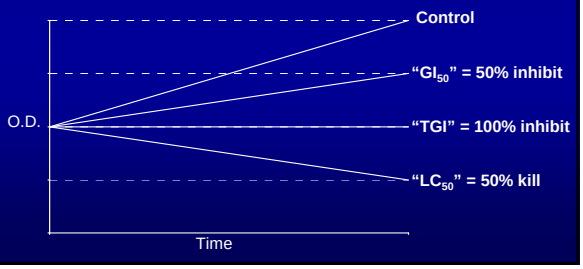
- Cell type specific agents
- Activity in solid tumors

Emerging Realities:

- Unique patterns of activity, cut across cell types
  AND
  Cell type selective patterns found
- Correlations of compound activity
  - relate to molecular “target” expression
  - generate hypothesis re: molecular target

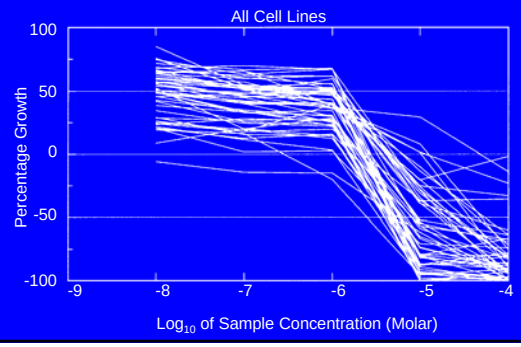
NCI IN VITRO CANCER CELL LINE SCREEN

- 60 cell lines
  (8 breast, 2 prostate, 8 renal, 6 ovary, 7 colon, 6 brain, 9 lung, 8 melanoma, 6 hematopoietic)
- 48 hr exposure; protein stain O.D.



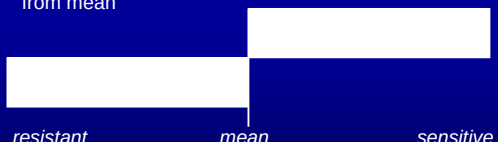
National Cancer Institute Developmental Therapeutics Program
Dose Response Curves

NSC: 643248-Q/2 ***(a rapamycin)*** | **Exp. ID:** 9503SC35-46



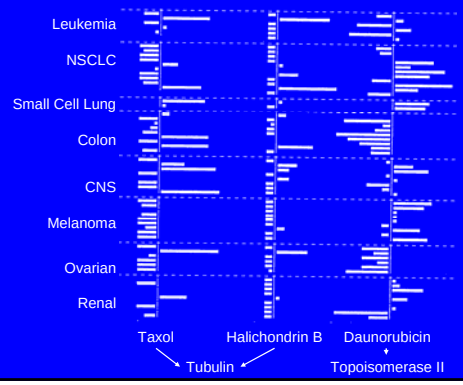
PATTERN RECOGNITION ALGORITHM: COMPARE

- Goal: COMPARE degree of similarity of a new compound to standard agents
- Calculate mean GI_{50}, TGI or LC_{50}
- Display behavior of particular cell line as deflection from mean



- Calculate Pearson correlation coefficient:
  1 = identity ; 0 = no correlation

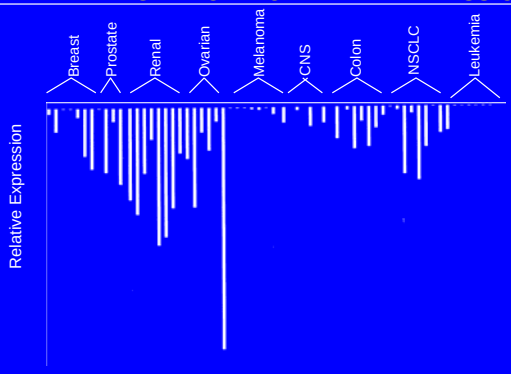
AGENTS WITH SIMILAR MECHANISMS HAVE SIMILAR MEAN GRAPHS



THE COMPARE ALGORITHM
Seed: Rubidazone

164011	1.000	Rubidazone
82151	0.921	Daunomycin
123127	0.915	Adriamycin
665934	0.891	Epipodophyllotoxin analogue
Discreet	0.880	Gyrase-To-TOPO analogue
Discreet	0.867	AMSA analogue
267469	0.865	Deoxydoxorubicin
305884	0.865	Acodazole HCL
665935	0.864	Epipodophyllotoxin analogue
668380	0.861	Azatoxin analogue
639659	0.854	Adriamycin analogue
644946	0.850	Epipodophyllotoxin analogue
254681	0.848	Daunomycin analogue
Discreet	0.847	Epipodophyllotoxin analogue
Discreet	0.843	Epipodophyllotoxin analogue
180510	0.842	Daunomycin analogue
Discreet	0.837	Epipodophyllotoxin analogue
Discreet	0.833	Gyrase-To-TOPO analogue

RELATIVE EGF RECEPTOR mRNA EXPRESSION

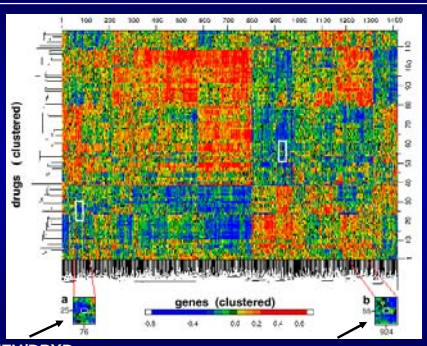


COMPARE ANALYSIS: EGF RECEPTOR

RANK	CORRELATION	CHEMICAL NAME
1	0.71	TGFα-PE40
2	0.66	Toxin-Δ53L, MW=43K
7	0.57	EGFR Tyrosine Kinase Inhibitor
88	0.43	EGFR Tyrosine Kinase Inhibitor

40,421 COMPOUNDS IN THE NCI DATABASE

DRUG TARGET CLUSTERINGS REVEAL CLUES TO MECHANISM



5FU/DPYD **L-Asparaginase/ASNS**

Nature Genetics 24: 236, 2000; http://dtp.nci.nih.gov

OUTLINE OF PRESENTATION

- General Introduction
- Definition of Drug Targets
- Generating Diversity
- Definition of Lead Structures
- ***Qualifying Lead for Transition to Early Trials***

GOALS OF PRECLINICAL DRUG STUDIES

Regulatory framework

- IND = "Investigational New Drug" application = approval by FDA to conduct human studies; main criterion : SAFETY AND LIKELY REVERSIBLE TOXICITY = allows *start* of Phase I trials
- NDA = "New Drug Application" = basis for sale to public; main criteria: SAFETY AND SOME MEASURE OF EFFICACY = *result* of Phase II/III trials

COMPONENTS OF AN IND

The goal of the pre-clinical process

- "Form 1571"
- Table of Contents
- Intro Statement / Plan
- Investigator Brochure
- Clinical Protocol
- Chemistry, Manufacture, Control
- Pharmacology/ Toxicology
- Prior Human Experience
- Additional Info - Data monitoring, Quality Assurance

OBJECTIVES OF PRECLINICAL PHARMACOLOGY STUDIES FOR ANTI-NEOPLASTIC DRUGS

- Development of Sensitive Analytical Methods for Drugs in Biological Fluids & Tissues
- Determine *In Vitro* Stability and Protein Binding
- Determine Pharmacokinetics in Rodents (& Dogs)
- Identification and Analysis of Metabolites
- Define Optimal Dose Schedule and Blood Sampling Times
- Define C_P and/or AUC with Efficacy, Safety & Toxicity
- Analog Evaluation - Determine Optimal Development Candidate

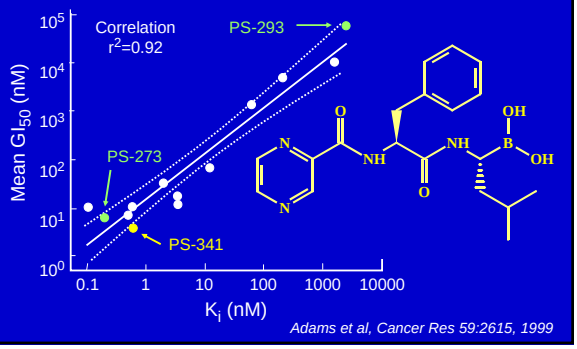
OBJECTIVES OF PRECLINICAL TOXICOLOGY STUDIES

- DETERMINE IN APPROPRIATE ANIMAL MODELS:
  - The Maximum Tolerated Dose (MTD)
  - Dose Limiting Toxicities (DLT)
  - Schedule-Dependent Toxicity
  - Reversibility of Adverse Effects
  - A Safe Clinical Starting Dose

FDA PRECLINICAL PHARMACOLOGY & TOXICOLOGY REQUIREMENTS: ONCOLOGY Rx

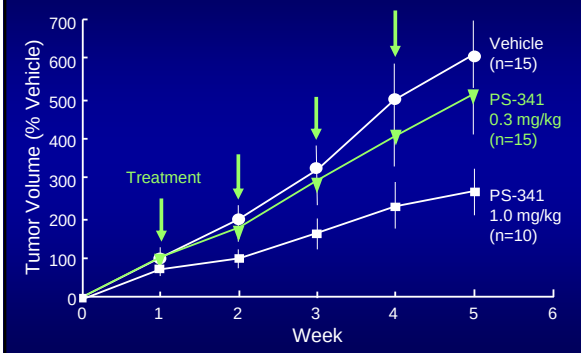
- DRUGS
  - Two Species - Rodent & Non-rodent
  - Clinical Route & Schedule
    - Follow NCI Guidelines
  - Pharmacokinetics - Optional
- BIOLOGICALS
  - Most Relevant Species
  - Clinical Route & Schedule

CORRELATION BETWEEN 20S PROTEASOME INHIBITORY POTENCY & GROWTH INHIBITION FOR 13 DIPEPTIDE BORONIC ACIDS



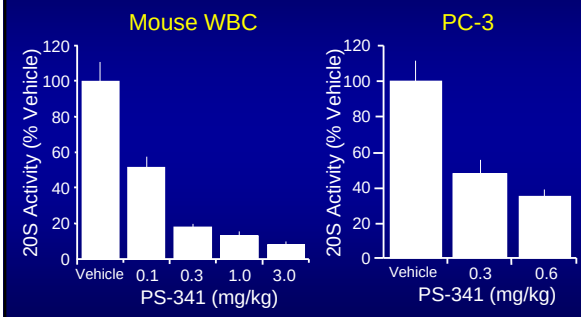
Adams et al, Cancer Res 59:2615, 1999

EFFECT OF PS-341 ON PC-3 TUMOR GROWTH IN MICE



Adams et al, Cancer Res 59:2615, 1999

EFFECT OF PS-341 ON 20S PROTEASOME ACTIVITY



Adams et al, Cancer Res 59:2615, 1999

PS-341: INTERSPECIES

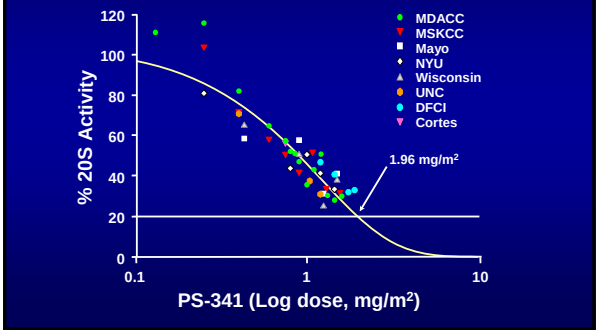
Q: Is the 'safe' dose in animals in the efficacy range for man?

Species	Dose (mg/kg)	Dose (mg/m^2)	% 20S Proteasome Inhibition*
Mouse	1.0	3.0	80
Rat	0.25	1.5	80
NHP	0.067	0.8	70

***In white blood cells at 1.0 h, post-dose**

Ref: Adams, *et al, Cancer Res* 59:2615, 1999

Ex Vivo Proteasome Activity: 1 Hour Post Treatment



ACKNOWLEDGEMENTS

NCI

J. Tomaszewski
M. Alley
M. Hollingshead / S. Stinson
J. Johnson
A. Monks / N. Scudiero
S. Bates
D. Zaharevitz / R. Gussio
S. Decker
R. Shoemaker / M. Currens

J. Adams
Millenium
J. Lazo
U. Pittsburgh